BREEDING PIERCE'S DISEASE RESISTANT WINEGRAPES

Principal Investigator:
Andrew Walker
Dept. of Viticulture and Enology
University of California
Davis, CA 95616
awalker@ucdavis.edu

Cooperating Staff:
Alan Tenscher
Dept. of Viticulture and Enology
University of California
Davis, CA 95616
actenscher@ucdavis.edu

Reporting Period: The results reported here are from work conducted September 2010 to October 2011.

ABSTRACT
We continue to make rapid progress breeding Pierce's disease (PD) resistant winegrapes. Aggressive vine training and selection for precocious flowering has allowed us to reduce the seed-to-seed cycle to two years. We are also using marker-assisted selection (MAS) for the PD resistance gene, *PdR1* (see companion report) to select resistant progeny as soon as seeds germinate. These two practices have greatly accelerated the breeding program and allowed us to produce four backcross generations with elite *V. vinifera* wine grape cultivars in 10 years. In Spring 2010, we planted about 2,000 97% *vinifera* seedlings with *PdR1*. We finished the evaluating the fruit quality of over 1,200 of these in September 2011, and produced a small-scale wine of one, 09333-178. We are preparing to greenhouse test the best of these to verify which have the highest level of resistance to PD prior to multiplication and grafting for larger scale field trials. We plan to release commercially useful varieties from populations at this 97% *vinifera* level. The resistance above is based on 8909-08, which has one of the two alleles, *PdR1b*, from the *Vitis arizonica/candicans* b43-17 resistance source. The other resistance allele, *PdR1a*, is in 8909-17 and we have advanced this resistance to the 94% *vinifera* level and have combined it with the *PdR1b* allele to determine whether resistance with both alleles is stronger. There is also strong resistance in b42-26 a form of *V. arizonica/ girdiana* form Baja California. b42-26's resistance is controlled by multiple genes, as opposed to the single gene resistance found in b43-17. We made crosses this year to advance the b42-26 resistance to the 87% *vinifera* level and have been surprised no only by the strength of resistance but also by the relatively large number of resistant progeny each generation. We are now re-evaluating its resistance markers to verify that it is not another form of *PdR1*. Finally, we evaluated the first set of about 50 accessions collected across the southwestern US for PD resistance. There were many with very strong resistance and these will be tested to verify that their resistance is different from b43-17's. This year's wine making also included wines made as blends with elite vinifera winegrapes, a likely use of our eventual releases. These selections could be used in severe PD hot spots and the fruit could be blended into the rest of the vineyard in a 25/75% ratio. We used Napa Valley (Oakville Station) Sauvignon blanc and Merlot with 07713-051 and 07355-075, respectively. We also made wine at the same scale with the Napa Sauvignon blanc and Merlot, and Davis Sauvignon blanc and Merlot (as growing region controls).

INTRODUCTION
The Walker lab is uniquely poised to undertake this important breeding effort, having developed rapid screening techniques for *Xylella fastidiosa* (*Xf)* resistance (Buzkan et al. 2003, Buzkan et al. 2005, Krivanek et al. 2005a 2005b, Krivanek and Walker 2005), and having unique and highly resistant *Vitis rupestris* x *V. arizonica* selections, as well as an extensive collection of southeastern grape hybrids, to allow the introduction of extremely high levels of *Xf* resistance into commercial grapes. We have selected progeny with *PdR1* from the b43-17 *V. arizonica*/*candicans* resistance source for fruit quality at the backcross 4 (BC4), 97% *vinifera* level. They are also undergoing greenhouse testing to verify their resistance and those with the highest levels of resistance will be prepared for small-scale winemaking this winter by grafting them onto Pierce's disease (PD) resistant rootstocks and planting six to eight vines sets on commercial spacing and trellising. We have made wine from vines that are 94% *vinifera* level from the same resistance background for two years. They have been very good and do have the hybrid flaws (blue purple color and herbaceous aromas and taste) that were prevalent in wines from the 87% *vinifera* level. There are two forms of *PdR1*, 8909-08 and 8909-17 – sibling progeny of b43-17 and they have different alleles of *PdR1*. These selections have been introgressed into a wide range of winegrape backgrounds over multiple generations, and resistance from southeastern United States (SEUS) species is being advanced in other lines. However, the resistance in these later lines is complex and markers have not yet been developed to expedite breeding.

OBJECTIVES
1. Breed Pierce's disease resistant winegrapes through backcross techniques using high quality *V. vinifera* winegrape cultivars and *Xf* resistant selections and sources characterized from our previous efforts.
2. Continue the characterization of *Xf* resistance and winegrape quality traits (color, tannin, ripening dates, flavor, productivity, etc) in novel germplasm sources, in our breeding populations, and in our genetic mapping populations.

RESULTS AND DISCUSSION

Objective 1

Table 1 presents the 2011 PD crosses made, although the list does not include crosses made to pyramid PD resistance with powdery mildew resistance. We used Nero d' Avola, a red wine grape from southern Italy and Sicily with very good color and tannin, and Pinot blanc, a Burgundian white wine grape to make crosses to increase our populations that contain PD resistance from 8909-08 (*PdR1b* from *V. arizonica/candicans* b43-17) at the 97% *vinifera* (backcross 4; BC4), and the 98.4% *vinifera* BC5 levels (**Table 1a**.). These crosses should generate over 500 seedlings of which half should have markers for, and resistance to, PD. These seedlings will add to our original planting of 97% *PdR1b* containing seedlings from our 2009 crosses (first fruit results are presented in **Table 3**).

There were 583 97% *vinifera PdR1b* containing seedlings planted in Spring 2011 from the 2010 crosses. These seedlings are 50% of their last *vinifera* parent and they largely express that last parent in their appearance and fruit quality. Those parents and the number of seedlings MAS for *PdR1b* that were planted include Barbera (263), Chardonnay (67), Muscat Blanc (31), and Riesling (222). If we combine the 2009-2011 *PdR1b* crosses, the last generation *vinifera* parents now number 11: Barbera, Cabernet Sauvignon, Chardonnay, Chenin blanc, Muscat blanc, Nero d'Avola, Pinot blanc, Pinot noir, Riesling, Sylvaner and Zinfandel.

In our greenhouse screening trials we always include b43-17 as a highly resistant standard to judge the effectiveness of the screen. In these screens we find that b43-17 is more resistant in terms of lower *Xf* levels (mean cfu/ml) in inoculated stems. These results prompted efforts to increase the durability or breadth of resistance by integrating resistance from other backgrounds and to combine both alleles of *PdR1* into one background. We have made the most progress with *PdR1b* from 8909-08, but we are now advancing populations with *PdR1a* from 8909-17 and exploring other facets of b43-17'S resistance. **Table 1b** presents crosses made with 75% *vinifera PdR1* selections back to *vinifera* to create mapping populations designed to examine the impact of minor genes associated with *PdR1*.

Resistance in *V. arizonica/girdiana* b42-26 tested as a quantitative multi-gene trait, which greatly suppresses *Xf*. Because resistance is quantitative backcross breeding is less effective. However, we continue to breed and map with this resistance (**Table 1c**) and have made our first crosses to combine b42-26 resistance to our advanced selections with *PdR1* (**Table 1d**). These efforts have been promoted by promising greenhouse screen results with b42-26 at the 75% *vinifera* level (**Table 2, group D**). Ultimately we want to combine these resistance sources to broaden and strengthen resistance. This Spring we broadened the wine quality background at the 87.5% *vinifera* level with crosses to F2-35 and Zinfandel.

Table 1. Pierce's disease resistant crosses made in 2011.

Resistant Type	*Vinifera* Parent\Grandparent of Resistant Type	*Vinifera* Types used in 2011 crosses	Estimated # of Seed
1a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny between 97% and 98.4% *V. vinifera* parentage. F2-35 is 100% *vinifera* cross of Cabernet Sauvignon x Carignane.			
07355-020	Petite Sirah\Cabernet Sauvignon	Nero d'Avola	260
07370-039	F2-35\Chardonnay	Nero d'Avola	220
09-331	Zinfandel\Petite Sirah	Nero d'Avola, Pinot blanc	315
1b. Monterrey *V. arizonica/candicans* resistance source (b43-17) to produce progeny with 75% *V. vinifera* parentage for the discovery of minor genes for PD resistance.			
09373-01	F2-35	08319-12 (Zinfandel selfed), Rosa Minna	70
04373-02	F2-35	Pinot blanc, Zinfandel	565
04373-22	F2-35	Pinot blanc, Zinfandel	1,025
1c. Crosses to the b42-26 *V. arizonica* resistance source to produce progeny that are 87.5% *vinifera* and 12.5% the resistance source.			
07344A-10	Grenache	F2-35	175
07344A-11	Grenache	Zinfandel	175
07344A-24	Grenache	F2-35	220
07344A-35	Grenache	F2-35	700
1d. Cross made to pyramid *PdR1b* b43-17 Monterrey *V. arizonica/candicans* and b42-26 *V. arizonica* resistance lines to produce progeny between 84% and 86% *vinifera*.			
09-331	Zinfandel\Petite Syrah	Grenache\F2-35	725
07355-020	Petite Sirah\Cabernet Sauvignon	Grenache\F2-35	175
07370-039	F2-35\Chardonnay	Grenache\F2-35	60

Table 2 details the greenhouse screening we have done in 2010-11. Groups A and H were tests of populations being pursued as PD resistant rootstocks. Although we screen our selections for the highest level of resistance and the lack of PD symptoms, there is a chance that *Xf* could move downwards into the rootstock and damage a susceptible rootstock. Thus, we are breeding rootstocks with *PdR1* to ensure that they will be equally resistant. We are also incorporating resistance to a broad range of nematodes and screening for good rooting and vineyard performance.

Group B was a test of 97% *vinifera PdR1b* selections from our 2009 populations. These 23 selections were made based on the presence markers for *PdR1* and their vigor and appearance. They were grafted and moved to our larger Y trellis on commercial spacings to get more fruit for small-scale winemaking. The greenhouse testing found that seven of these selections had relatively high *Xf* levels even though they had *PdR1* markers. We have seen this level of resistance dilution in these later generations. However, the rest had high levels of resistance. This group included 09333-178, which was fruitful enough to allow very small wine making this year (**Table 4a-c**) and is very promising (**Figure 3**). The rest will be ready for small-scale winemaking in 2012 and we hope to choose selections for field testing from them. We have many more selections from these populations that will be evaluated when they fruit more reliably next year.

The Groups C, D, F and G provided screening results for our genetic mapping efforts with sources of PD resistance other than b43-17. Group G was the first of several tests examining our southwestern *Vitis* collections to identify additional sources of PD resistance. These efforts are important, as we want to broaden resistance by combining multiple resistance sources. The results of this screen are pending but they include 54 accessions and are elaborated in our companion report on the genetic mapping of *Xf* resistance. Screening results with b42-26 suggest that a major resistance locus may have a greater impact than previously thought and that resistance is not only strong, but more individuals are resistant than expected. Mapping studies are examining these progeny to determine whether this resistance is another form of *PdR1*. The 2010 crosses included several populations with the most resistant b42-26 progeny crossed to Cabernet Sauvignon, Carignane and Chardonnay, which resulted in 214 seedlings that were planted this Spring. The *V. shuttleworthii* 'Haines City' resistance source was tested at the 75% *vinifera* level in Group F. Only nine of the 97 tested progeny were resistant. However, they were strongly resistant so we will continue to advance this population to the next generation, although fewer resistant progeny are being detected in each successive generation.

Group E tested progeny from crosses to combine advanced lines of both alleles of *PdR1* (a and b from 8909-17 and 8909-08, respectively). We are waiting for MAS results to verify which allele is in which seedling. The data will be very interesting and help determine whether stronger resistance is achieved with both alleles of the homozygous resistant b43-17. This Spring we planted 180 progeny with *PdR1a* at the 94% *vinifera* level, which should begin fruiting in 2012. Advanced selections from this population will be crossed to 97% *vinifera PdR1b* selections, resulting in individuals with both alleles.

Table 2. Groups of plants greenhouse screened for *Xf* resistance.

Group	Genotypes	# Genotypes	Inoculation Date	ELISA Sample Date	Resistance Source(s)
A	08 PD Rootstocks & Recombinants	22	7/15/10	10/14/10	F8909-08
B	97% *vinifera*, Y-trellis	23	7/26/10	11/23/10	F8909-08
C	05347 Mapping	122	9/23/10	1/6/11	b42-26
D	07344A, 07744RT, 2010 Parents	79	11/9/10	2/10/11	b42-26, b40-14, b43-17
E	*PdR1a* & *PdR1b* together	122	1/13/11	5/3/11	b43-17
F	Haines City & Supplemental	97	3/24/11	7/12/11	*V. shuttleworthii*
G	*V. arizonica* and southwestern *Vitis*	54	5/12/11	8/11/11	*V. arizonica* accessions
H	PD Rootstocks Adv. Selections	15	6/14/11	9/15/11	F8909-08

Objective 2

Table 3 presents results of the 2011 evaluation of the 97% *vinifera PdR1b* progeny fruit. This was the first year that our most advanced material fruited. The table lists the progeny by the last *vinifera* parent in the crosses – the progeny are 50% of that parent. The first row of the table presents the number of crosses made with each parent, followed by the number of seedling progeny tested. The number of precocious progeny is next – these are the seedlings that flowered this year. We will evaluate the seedlings that did not fruit this year in 2012. We then selected based on flavor and appearance over two selection passes (cuts) and advanced as many as possible to juice and berry evaluations. Seventy-seven seedlings made the second cut and berry evaluations were made on 65 of these (the other 12 were very early and will have to be tested next year). Based on cluster and berry size, and juice flavor and color, 23 were selected as the best (**Figures 1 and 2**). The entire set of 77 will be greenhouse tested this Winter to identify those with the strongest resistance to *Xf* before we begin propagating the best for multiple vine testing and small-scale wine making. Hundreds of more progeny from the 2009 97% *vinifera PdR1b* crosses will begin fruiting next year. There should be many more advanced selections available from these evaluations. In addition, the first of the progeny from the 2010 97% *vinifera PdR1b* crosses will begin fruiting.

Table 3. 97% *vinifera PdR1* 2009 cross seedlings evaluated for fruit quality for the first time in 2011.

Evaluation Level	*Vinifera* Parent							
	Cab Sauv	Chard	Chenin blanc	Pinot noir	Riesling	Sylvaner	Zinfandel	Total
# Crosses	7	4	6	3	1	3	4	28
# Seedlings (sdlg)	489	309	517	107	11	218	217	1868
# Precocious Sdlg	313	216	366	74	10	125	174	1278
# Sdlg making first cut	62	76	77	22	6	40	61	344
# Sdlg making 2nd cut	9	25	13	3	2	4	21	77
# Sdlg juice/berry evaluation	9	21	11	4	2	3	15	65
# Sdlg elite rating post juice/berry evaluation	5	5	4	1	1	-	7	23

Figure 1. Juice samples from 97% *vinifera PdR1b* selections.

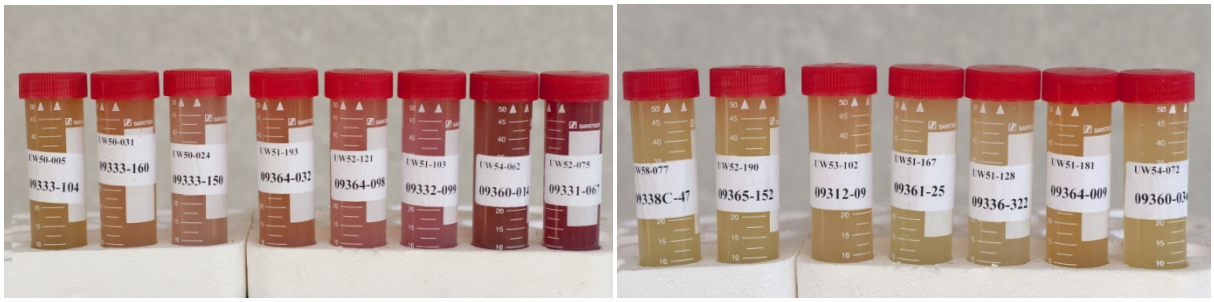

Figure 2. Range of juice color intensity from red and white 97% *vinifera PdR1b* selections.

Figure 3. 09333-178, the first 97% *vinifera PdR1b* selection used for small scale wine making in 2011.

We made 15 wine lots in 2011. Seven were from *PdR1* progeny (two at the 87% *vinifera* level, four at the 94% level and one at the 97% level) and included Blanc du Bois and Lenoir as our "state of the art" PD resistant southeastern US standards. We also made blended wines this year in anticipation of the likely use of many of our eventual releases. These selections could be used in severe PD hot spots and the fruit could be blended into the rest of the vineyard in a 25/75% ratio. We used Napa Valley (Oakville Station) Sauvignon blanc and Merlot with 07713-051 and 07355-075, respectively. We also made wine at the same scale with the Napa Sauvignon blanc and Merlot, and Davis Sauvignon blanc and Merlot (as growing region controls).

Table 4a-c provides the details of the vine, fruit and juice characteristics for the seven *PdR1* wine lots made in 2011. Two of the favorites from the 87% *vinifera* level were included; two 94% *vinifera* level selections that we made wine from last year, and two new 94% *vinifera* level selections; and our first effort at the 97% *vinifera* level, 09333-178.

Table 4a 2011 PD resistant wine lots background and fruit characteristics

Genotype	Parentage	% *vinifera*	2011 Bloom Date	Berry Color	Berry Size (g)	Avg Cluster Wt. (g)	Ripening Season	Prod 1=v low, 9=v high
07329-31	U0505-01 x Chardonnay	94%	5/24/11	B	1.0	136	mid	5
07355-48	U0505-01 x Petite Sirah	94%	5/24/11	B	1.1	262	mid	6
07355-75	U0505-01 x Petite Sirah	94%	5/27/11	B	1.1	245	late	7
07713-51	F2-35 x U0502-48	94%	5/19/11	W	1.2	229	early	8
09333-178	07355-020 x Chardonnay	97%	5/24/11	B	1.3	168	mid-late	5
U0502-20	A81-138 x Chardonnay	87.5%	5/21/11	W	1.1	183	early-mid	7
U0502-38	A81-138 x Chardonnay	87.5%	5/19/11	B	1.0	358	early	7
Lenoir	*V. aestivalis* hybrid	<50%	6/2/11	B	1.3	157	late	6

Table 4b 2011 PD wine lot juice chemical analysis. Data from Cab. Sauvignon, Pinot noir and Lenoir are form previous vintages for comparison.

Genotype	°Brix	TA (g/L)	pH	L-malic acid (g/L)	potassium (mg/L)	YAN (mg/L, as N)	catechin (mg/L)	tannin (mg/L)	Total antho-cyanins (mg/L)
07329-31	29.2	6.6	3.63	2.05	1810	548	2	395	1160
07355-48	26.2	5.9	3.54	1.89	1850	213	4	480	1624
07355-75	27.2	6.8	3.52	1.98	2050	324	<1	558	1397
07713-51	24.1	5.8	3.50	1.97	1540	210	-	-	-
09333-178	25.1	5.0	3.67	1.17	1800	265	61	328	595
U0502-20	23.9	6.9	3.52	2.92	1660	419	-	-	-
U0502-38	27.9	9.0	3.58	6.36	2570	449	18	737	1346
Cab. Sauvignon	24.9	6.2	3.65	2.19	2460	227	59	250	404
Pinot noir	26.5	4.9	3.83	2.43	2190	279	321	842	568
Lenoir	24.8	12.1	3.22	7.03	2240	183	186	268	2486

Table 4c 2011 PD wine lot berry sensory analysis. Skin tannin, seed color and seed tannin rated from 1 = low to 4 = high.

Genotype	Juice Hue	Juice Intensity	Juice Flavor	Skin Flavor	Skin Tannin	Seed Color	Seed Flavor	Seed Tannin
07329-31	pink-red	medium	fruity-stemmy	currant	1	4	woody	2
07355-48	red-pink	light	cherry jam	cassis	4	3	wood, astringent	2
07355-75	red	dark	jammy	prune	1	3	warm, spicy	1
07713-51	gold, sl brwn	dark	floral, melon	herbal	3	3	spicy, mildly hot	3
09333-178	orange	light	fruity, red apple	black plum	1	3	woody, sl bitter	2
U0502-20	green-brwn	medium	grn apple, melon	neutral	1	3	sl spicy, sl bitter	4
U0502-38	pink-red	med-dark	cherry, berry	plum	2	3	woody, ripe	1
Lenoir	red	med-dark	mildly fruity	fruity	1	4	hot	1

CONCLUSIONS

Strong progress continues to made breeding PD resistant winegrapes. We evaluated our first fruit at the 97% *vinifera PdR1* resistance level and were even able to get enough fruit from one selection to make a small-scale wine. We are advancing other forms of PD resistance to later generations with the goal of combining multiple resistance sources to broaden resistance. These include the other allele of *PdR1*, resistance from b42-26, and resistance from *V. shuttleworthii* 'Haines City'. We made small-scale wines again this year at the 87.5, 94 and 97% *vinifera* levels. We also made blended wines with a white 94% *vinifera* selection (07713-051) with Napa Valley Sauvignon blanc (25/75%) and a red 94% *vinifera* selection (07355-075) with Napa Valley Merlot to mimic how these selections might be used in North Coast PD hot spots.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board. Additional support from the Louis P. Martini Endowed Chair in Viticulture is also gratefully acknowledged.